

**WEST****Generate Collection****Search Results - Record(s) 1 through 1 of 1 returned.** **1. Document ID: US 5543312 A**

L10: Entry 1 of 1

File: USPT

Aug 6, 1996

DOCUMENT-IDENTIFIER: US 5543312 A

TITLE: *Pastuerella haemolytica* glycoprotease gene and the purified enzyme

## BSPR:

The leukotoxin is another secreted protein of *P. haemolytica* A1, for which the nucleotide sequence and the regulation of leukotoxin expression has been described, Lo et al. 1987. "Nucleotide Sequence of the Leucotoxin Genes of *Pasteurella haemolytica* A1." Infect. Immun.; Strathdee et al. 1989a, "Cloning, nucleotide sequence, and characterization of genes encoding the secretion function of the *Pasteurella haemolytica* leucotoxin determinant." J. Bacteriol; Strathdee et al 1989b. The leukotoxin determinant is composed of four contiguous genes lktCABD encoded on the same DNA strand where LktA is the structural gene for the leukotoxin (LktA), while proteins encoded by lktC functions in the activation of leukotoxin (LktA), while proteins encoded by lktB and lktD are involved in the secretion of leucotoxin. It possible that glycoprotease has a similar mode of activation as the leukotoxin, which could explain the lower specific activity of the enzyme expressed in *E. coli*. An examination of the amino terminus of the glycoprotease from pGPI shows no pattern similar to the conventional signal sequences predicted for a number of secreted proteins characterized Michaelis et al. 1982. "Mechanism of incorporation of cell envelope proteins in *Escherichia coli*." Ann. Rev.; Silhavy et al. 1988. "Mechanism of protein localization." Microbiol. Rev.; Von Heijne, G. 1983. "Patterns of amino acids near signal sequence cleavage sites." Eur. J. Biochem. Since the glycoprotease is normally secreted from *P. haemolytica* A1, an alternative secretory mechanism not involving an amino-terminus signal may be utilized, as reported for the leukotoxin, Strathdee et al. 1989. "Cloning, nucleotide sequence, and characterization of genes encoding the secretion function of the *Pasteurella haemolytica* leukotoxin determinant". J. Bacteriol.

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**WEST****End of Result Set** **Generate Collection**

L10: Entry 1 of 1

File: USPT

Aug 6, 1996

US-PAT-NO: 5543312

DOCUMENT-IDENTIFIER: US 5543312 A

TITLE: *Pastuerella haemolytica* glycoprotease gene and the purified enzyme

DATE-ISSUED: August 6, 1996

INT-CL: [6] C12N 1/21, C12N 9/52, C12N 15/57, C12P 21/00

US-CL-ISSUED: 435/220; 435/240.2, 435/252.3, 435/320.1, 536/23.2, 536/24.32

US-CL-CURRENT: 435/220; 435/252.3, 435/320.1, 536/23.2, 536/24.32FIELD-OF-SEARCH: 536/23.2, 536/24.32, 536/24.3, 435/252.3, 435/240.2, 435/320.1,  
435/6, 435/220

**Expression of the *Pasteurella haemolytica* leukotoxin is inhibited by a locus that encodes an ATP-binding cassette homolog [published erratum appears in Infect Immun 1993 Dec;61(12):5431]**

Highlander SK; Wickersham EA; Garza O; Weinstock GM  
Department of Microbiology and Immunology, Baylor College of Medicine, Houston, Texas 77030.

Infection and immunity (UNITED STATES) Sep 1993, 61 (9) p3942-51,  
ISSN 0019-9567 Journal Code: GO7

Contract/Grant No.: RR-05425, RR, NCRR

Languages: ENGLISH

Document type: JOURNAL ARTICLE

JOURNAL ANNOUNCEMENT: 9312

Subfile: INDEX MEDICUS

Multicopy and single-copy chromosomal fusions between the *Pasteurella haemolytica* leukotoxin regulatory region and the *Escherichia coli* beta-galactosidase gene have been constructed. These fusions were used as reporters to identify and isolate regulators of leukotoxin expression from a *P. haemolytica* cosmid library. A cosmid clone, which inhibited leukotoxin expression from multicopy and single-copy protein fusions, was isolated and found to contain the complete leukotoxin gene cluster plus additional upstream sequences. The locus responsible for inhibition of expression from leukotoxin -beta-galactosidase fusions was mapped within these upstream sequences, by transposon mutagenesis with Tn5, and its DNA sequence was determined. The inhibitory activity was found to be associated with a predicted 440-amino-acid reading frame (lapA) that lies within a four-gene arginine transport locus. LapA is predicted to be the nucleotide-binding component of this transport system and shares homology with the Clp family of proteases.

Tags: Support, Non-U.S. Gov't; Support, U.S. Gov't, P.H.S.

Descriptors: Adenosinetriphosphatase--Genetics--GE; \*Bacterial Toxins--Genetics--GE; \*Chromosome Mapping; \*Exotoxins--Genetics--GE; \*Heat-Shock Proteins--Genetics--GE; \* *Pasteurella haemolytica*--Pathogenicity--PY; \*Serine Endopeptidases--Genetics--GE; Amino Acid Sequence; Base Sequence; DNA, Bacterial--Chemistry--CH; Genes, Bacterial; Lac Operon; Molecular Sequence Data; Multigene Family; *Pasteurella haemolytica*--Genetics--GE; Recombinant Fusion Proteins--Genetics--GE

Molecular Sequence Databank No.: GENBANK/M59210

CAS Registry No.: 0 (leukotoxin); 0 (Bacterial Toxins); 0 (DNA, Bacterial); 0 (Exotoxins); 0 (Heat-Shock Proteins); 0 (Recombinant Fusion Proteins)

Enzyme No.: EC 3.4.21 (Serine Endopeptidases); EC 3.4.21.53 (endopeptidase La); EC 3.6.1.3 (Adenosinetriphosphatase)

Gene Symbol: lapA

4/9/60 (Item 2 from file: 155)

DIALOG(R) File 155: MEDLINE(R)

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07630543 93273518

**Enhancement of neutrophil-mediated injury to bovine pulmonary endothelial cells by *Pasteurella haemolytica* leukotoxin.**

Maheswaran SK; Kannan MS; Weiss DJ; Reddy KR; Townsend EL; Yoo HS; Lee BW ; Whiteley LO

Department of Veterinary Pathobiology, College of Veterinary Medicine, University of Minnesota, St. Paul 55108.

Infection and immunity (UNITED STATES) Jun 1993, 61 (6) p2618-25,  
ISSN 0019-9567 Journal Code: GO7

Languages: ENGLISH

Document type: JOURNAL ARTICLE

JOURNAL ANNOUNCEMENT: 9309

Subfile: INDEX MEDICUS

In this study, we used an in vitro coculture system to determine which virulence factor from *Pasteurella haemolytica* A1 was responsible for augmenting bovine polymorphonuclear neutrophil (PMN)-mediated killing of bovine pulmonary artery endothelial cells (BPAEC). A <sup>51</sup>Cr release cytotoxicity assay was used as a measure of BPAEC killing. The mechanisms

**Field-trial evaluation of a *Pasteurella* vaccine in preconditioned and nonpreconditioned lightweight calves.**

Kadel WL; Chengappa MM; Herren CE

American journal of veterinary research (UNITED STATES) Sep 1985, 46  
(9) p1944-8, ISSN 0002-9645 Journal Code: 40C

Languages: ENGLISH

Document type: JOURNAL ARTICLE

JOURNAL ANNOUNCEMENT: 8601

Subfile: INDEX MEDICUS

A field-trial evaluation confirmed the efficacy of a *Pasteurella* vaccine as a means of preventing bovine pneumonia. The vaccine was comprised of streptomycin-dependent *Pasteurella multocida* (type A:3) and *Pasteurella haemolytica* (type 1). Vaccinal efficacy was defined in terms of greater body weight gains, less severe clinical signs of pneumonia, and smaller death rates as compared with the same factors in nonvaccinated calves. During the 50-day trial, vaccinated calves gained weight faster than did nonvaccinated calves ( $P = 0.05$ ). Economic advantage was not found for administering a booster dose of the vaccine ( $P = 0.25$ ). Nonpreconditioned nonvaccinated calves made greater dollar profits than did preconditioned nonvaccinated calves ( $P = 0.16$ ). A comparison of all preconditioned calves with all nonpreconditioned calves revealed that illness and death losses were less in the preconditioned calves ( $P = 0.07$ ). An evaluation of the cost vs benefit factors revealed significant advantages for administering 1 dose of vaccine of \$19.08 for a preconditioned calf ( $P = 0.006$ ) and of \$11.39 for a nonpreconditioned calf ( $P = 0.05$ ). The data indicated that there was no economic advantage for preconditioning and that the greatest economic gain was made by the vaccinated nonpreconditioned calves.

Tags: Animal; Comparative Study

**Comparative study of vaccines in preventing respiratory disease in beef calves after weaning.**

Weiblen R; Woods GT; Mansfield ME; Crandell RA

Research communications in chemical pathology and pharmacology (UNITED STATES) Feb 1982, 35 (2) p303-24, ISSN 0034-5164 Journal Code: R62

Languages: ENGLISH

Document type: JOURNAL ARTICLE

JOURNAL ANNOUNCEMENT: 8208

Subfile: INDEX MEDICUS

Vaccines against infectious bovine rhinotracheitis (IBR), bovine viral diarrhea (BVD), bovine parainfluenza-3 (PI-3), and bacterin against *Pasteurella hemolytica* and *P. multocida* were studied to determine their effectiveness when given 30 days before weaning in preventing respiratory disease in beef calves after weaning. A total of 310 calves, 6 to 8 months old, were divided into 3 groups. Group I consisted of 51 calves vaccinated with a temperature sensitive mutant vaccine intranasally (IBR-PI-3 TSV2). The 56 calves in Group 2 received another intranasal vaccine containing a modified live virus (IBR-PI-3 IP). Calves in both groups received a modified live bovine virus diarrhea (BVD) virus vaccine 30 days before weaning, and a bacteria containing Clostridium chauvei-septicum, *Pasteurella hemolytica*, and *P. multocida*. The bacterin was given twice, 2 weeks apart before weaning. The remaining 207 calves were used as unvaccinated controls. All calves were treated at least once after weaning for clinical respiratory illness. The only virus isolated was PI-3. The serologic response to the viral vaccines was good. There was some doubt as to the effectiveness of the PI-3 component because vaccinated calves were affected by this virus after weaning. Further studies are needed on PI-3 virus vaccine and the most effective vaccination schedule. Vaccination at the time of weaning may have been helpful in the present experiment in preventing clinical disease.

**Cloning, nucleotide sequence, and characterization of genes encoding the secretion function of the *Pasteurella haemolytica* leukotoxin determinant.**

**Strathdee CA ; Lo RY**

Department of Microbiology, University of Guelph, Ontario, Canada.

Journal of bacteriology (UNITED STATES) Feb 1989, 171 (2) p916-28,

ISSN 0021-9193 Journal Code: HH3

Languages: ENGLISH

Document type: JOURNAL ARTICLE

JOURNAL ANNOUNCEMENT: 8905

Subfile: INDEX MEDICUS

The structural gene of the *Pasteurella haemolytica* leukotoxin determinant is highly homologous to that of the *Escherichia coli* hemolysin determinant, which also encodes a specialized set of genes involved in the secretion of the hemolysin. In this report, we describe the cloning and nucleotide sequence of the analogous secretion genes from *P. haemolytica* which make up the remainder of the leukotoxin determinant. The secretion genes were cloned directly from the *P. haemolytica* chromosome to form the recombinant plasmid pPH5B. By subcloning the secretion genes together with the leukotoxin structural gene, the cloned leukotoxin determinant was reconstructed on a single plasmid, pLKT52, which directs the synthesis of active leukotoxin to the culture supernatant when expressed in *E. coli*. DNA sequence analysis showed the presence of two secretion genes, designated lktB and lktD in order of their genetic organization, which code for proteins of 79.7 and 54.7 kilodaltons, both of which were detected when pLKT52 was expressed in *E. coli* minicells. The lktB and lktD genes were found to be highly homologous to the hlyB and hlyD secretion genes of the hemolysin determinant, and the predicted LktB-HlyB and LktD-HlyD proteins were 90.5 and 75.6% homologous. Nucleotide sequence homology between the leukotoxin and hemolysin determinants was limited to the C, A, B, and D coding regions, although the presence of similar transcriptional terminators in the A-B intercistronic region is suggestive of a similar transcriptional organization. On the basis of these data, we hypothesize that the two determinants share a common evolutionary history and are prototypes for a widely disseminated family of virulence factors, the RTX cytotoxins.

Tags: Support, Non-U.S. Gov't

Nucleotide sequence of the leukotoxin genes of *Pasteurella haemolytica*  
A1.

Lo RY; Strathdee CA ; Shewen PE

Infection and immunity (UNITED STATES) Sep 1987, 55 (9) p1987-96,

ISSN 0019-9567 Journal Code: GO7

Languages: ENGLISH

Document type: JOURNAL ARTICLE

JOURNAL ANNOUNCEMENT: 8712

Subfile: INDEX MEDICUS

A 4.4-kilobase-pair DNA fragment coding for the leukotoxin of *Pasteurella haemolytica* A1 has been isolated, and its nucleotide sequence has been determined. Two open reading frames, designated lktC and lktA, coding for proteins of 19.8 and 101.9 kilodaltons, respectively, were identified. Expression of the two genes in minicell-labeling experiments resulted in the production of the predicted proteins LKTC and LKTA. By using an antiserum against the soluble antigens of *P. haemolytica* A1 in Western blot (immunoblot) analysis of total cellular proteins from the *Escherichia coli* clones, LKTA was identified as an additional antigenic protein. Results from subcloning of the DNA fragment suggested that expression from both lktC and lktA is required for leukotoxin activity, indicating that the leukotoxin of *P. haemolytica* A1 is encoded by two genes. A comparison of the organization and the DNA sequence of the leukotoxin genes with those of the *E. coli* alpha-hemolysin genes showed a significant degree of homology between the two loci. This analysis suggested that the leukotoxin genes of *P. haemolytica* A1 and the *E. coli* alpha-hemolysin genes may have evolved from a common ancestor and that the two toxins may share similar activities or functional domains or both.

Tags: Support, Non-U.S. Gov't

**Characterization of bovine pulmonary and serum antibody responses after parenteral or intrapulmonary vaccination with live *Pasteurella haemolytica*.**

McBride JW; Corstvet RE; Paulsen DB; McClure JR; Enright FM  
Department of Veterinary Science, Louisiana State University Agricultural Center, Baton Rouge, USA.

Comparative immunology, microbiology and infectious diseases (ENGLAND)  
Feb 1996, 19 (2) p99-115, ISSN 0147-9571 Journal Code: DNN

Languages: ENGLISH

Document type: JOURNAL ARTICLE

JOURNAL ANNOUNCEMENT: 9612

Subfile: INDEX MEDICUS

Pulmonary and serum antibody responses were evaluated in eight calves vaccinated [four intrapulmonary-right diaphragmatic lobe (IP) and four subcutaneous (SC)] with *Pasteurella haemolytica* A1 (Ph-1) impregnated agar beads and eight respective sham-vaccinated calves. Experimental and sham groups were challenged in both diaphragmatic lobes with Ph-1 34-37 d after vaccination (DAV) and necropsied 6 d after challenge (DAC; 40-43 DAV). IgG antibodies contained in fluids from the diaphragmatic lobes of vaccinated calves had different patterns of antigen specificity compared with IgG antibodies in analogous sera. Using ELISA, anti-Ph-1 IgA and IgG antibody concentrations were significantly higher ( $P < 0.05$ ) in lung lavage fluids from the IP group before and after challenge compared to the SC and sham groups. The IP and SC groups developed IgA, IgG and IgM antibody titers in nonvaccinated lung lobes after vaccination and challenge. The IP and SC groups exhibited significantly ( $P < 0.05$ ) smaller pulmonary lesions than the sham groups and pulmonary IgG and IgA antibodies were associated with increased protection.

Tags: Animal; Comparative Study; Support, Non-U.S. Gov't

**Passive immunity to *Pasteurella haemolytica* A1 in dairy calves:  
effects of preparturient vaccination of the dams.**

Hodgins DC; Shewen PE

Department of Veterinary Microbiology and Immunology, Ontario Veterinary College, University of Guelph.

Canadian journal of veterinary research (CANADA) Jan 1994, 58 (1)  
p31-5, ISSN 0830-9000 Journal Code: CKL

Languages: ENGLISH

Document type: JOURNAL ARTICLE

JOURNAL ANNOUNCEMENT: 9407

Subfile: INDEX MEDICUS

Dairy cows from five herds were assigned to receive a commercial *Pasteurella haemolytica* vaccine or no vaccine at all, administered at six and three weeks before parturition. Vaccination was associated with increased leukotoxin neutralizing serum antibody titers in the dams ( $p < 0.001$ ), and with increased titers in colostrum ( $p < 0.001$ ). Vaccination of dams also had a significant association with increased passive leukotoxin neutralizing antibody titers in their calves ( $p < 0.001$ ). Vaccination was also associated with increased indirect agglutinating antibody titers in serum of the dams ( $p < 0.001$ ). In the analysis of agglutinating antibody titers in colostral whey the interaction "vaccination\*herd" was found to be significant ( $p < 0.001$ ), indicating that the effects of vaccination on colostral titers were not consistent from herd to herd. The analysis was repeated, stratifying by herd. Vaccination was associated with increased agglutinating antibody titers in colostrum ( $p < 0.05$ ) in three herds of the five in the study. In two of these three herds there were significant increases in passive neonatal titers associated with vaccination. In the remaining herd the mean IgG1 level in the calves was consistent with failure of passive transfer of immunoglobulins ( $IgG1 < 8.0 \text{ g/L}$ ). These results suggest that preparturient vaccination of dairy cows can induce modest increases in passive antibody titers to antigens of *Pasteurella haemolytica* in their calves, but the antigen of interest and the population being studied can affect the outcome.

Tags: Animal; Female; Support, Non-U.S. Gov't

**Disturbances in ex vivo vascular smooth muscle responses following exposure to *Pasteurella haemolytica* vaccines.**

Weekley LB; Eyre P

Department of Biomedical Sciences, Virginia-Maryland Regional College of Veterinary Medicine, Virginia Polytechnic Institute and State University, Blacksburg 24060.

Journal of veterinary pharmacology and therapeutics (ENGLAND) Dec 1993,  
16 (4) p446-53, ISSN 0140-7783 Journal Code: KCP

Languages: ENGLISH

Document type: JOURNAL ARTICLE

JOURNAL ANNOUNCEMENT: 9406

Subfile: INDEX MEDICUS

Rats were vaccinated with saline (control) or one of the two commercially available *Pasteurella haemolytica* vaccines Presponse or Precon-PH. Animals were killed 3 days later and thoracic aorta removed for evaluation of the ex vivo biophysical responses to carbachol (CCh). In some experiments, vascular endothelium was mechanically removed. Vaccination of rats impairs the endothelial-dependent relaxation to CCh. In vessels with endothelium removed, the contractile response to CCh is converted into a relaxation following vaccination. Treatment of endothelial-denuded vascular rings ex vivo with methylene blue, a guanylate cyclase inhibitor, reduced the vaccination effect. Treatment of vascular rings with the superoxide dismutase inhibitor diethyldithiocarbamate, impairs the relaxant response of de-endothelialized vessels to CCh in Presponse vaccinated rats while enhancing the relaxation response of vessels from Precon-PH vaccinated rats. De-endothelialized vessels from vaccinated rats, but not control rats, relaxed in the presence of N-monomethyl-L-arginine (L-NMMA), a competitive inhibitor of nitric oxide synthetase. Furthermore, in the presence of L-NMMA, the relaxant response to CCh is significantly enhanced by Precon-PH but not Presponse. The normal relaxant response to hydrogen peroxide is converted into a contraction following vaccination. Results suggest that exposure to commercially available *P. haemolytica* vaccines alters vascular smooth muscle reactivity to CCh and that several independent pathways may be altered.

Development of a combined clostridial and *Pasteurella haemolytica*  
vaccine for sheep.

Wells PW; Robinson JT; Gilmour NJ; Donachie W; Sharp JM  
Veterinary record (ENGLAND) Mar 17 1984, 114 (11) p266-9, ISSN  
0042-4900 Journal Code: XBS

Languages: ENGLISH

Document type: JOURNAL ARTICLE

JOURNAL ANNOUNCEMENT: 8407

Subfile: INDEX MEDICUS

The efficacy of a multicomponent clostridial vaccine containing *Pasteurella haemolytica* antigens was tested in specific pathogen free or conventionally reared lambs exposed to experimental infection with *P haemolytica* serotypes A1, A2 or A6. In four experiments assessment was based upon the findings of clinical, pathological and bacteriological examinations. Three experiments carried out in conventionally reared lambs demonstrated protection against challenge infection with *P haemolytica* serotypes A1, A2 and A6 in vaccinated lambs. However, the inconsistency of the disease induced in these experiments emphasised the need to perform definitive studies in specific pathogen free conditions. The final experiment was carried out with specific pathogen free lambs and confirmed the efficacy of the multicomponent clostridial vaccine containing *P haemolytica* antigen in protecting against the effects of infection with *P haemolytica* serotype A6. In addition, this experiment indicated that the inclusion of several components in a vaccine did not affect the efficacy of an individual antigenic component.

Tags: Animal

Bovine pneumonic pasteurellosis: effect of culture age of *Pasteurella* la  
haemolytica used as a live vaccine.

Confer AW; Panciera RJ; Corstvet RE; Rummage JA; Fulton RW  
American journal of veterinary research (UNITED STATES) Dec 1984, 45  
(12) p2543-5, ISSN 0002-9645 Journal Code: 40C

Languages: ENGLISH

Document type: JOURNAL ARTICLE

JOURNAL ANNOUNCEMENT: 8505

Subfile: INDEX MEDICUS

Five experiments were conducted that compared aerosol immunization of calves with live *Pasteurella haemolytica* from logarithmic (6 hour) or stationary (20 to 22 hour) phase cultures. Calves were challenge exposed by transthoracic injection with *P haemolytica*. In 4 experiments, calves inoculated with 6-hour cultures had slightly lower mean lesion scores (indicating greater resistance to challenge exposure) than those inoculated with 20- to 22-hour cultures. High antibody titers, as detected by a quantitative fluorometric immunoassay or the indirect hemagglutination test, correlated directly with lung resistance (based on lesion scores) regardless of the age of the culture used as the immunogen.

Tags: Animal; Female; Male

Descriptors: Bacterial Vaccines--Immunology--IM; \*Cattle Diseases  
--Immunology--IM; \**Pasteurella* --Immuno

Bovine pneumonic pasteurellosis: effect of vaccination with live  
*Pasteurella* species.

Panciera RJ; Corstvet RE; Confer AW; Gresham CN

American journal of veterinary research (UNITED STATES) Dec 1984, 45

(12) p2538-42, ISSN 0002-9645 Journal Code: 40C

Languages: ENGLISH

Document type: JOURNAL ARTICLE

JOURNAL ANNOUNCEMENT: 8505

Subfile: INDEX MEDICUS

Experimental bovine pneumonic pasteurellosis was induced in beef calves by a transthoracic challenge exposure with *Pasteurella haemolytica* serotype 1 or P multocida type 3. Challenge exposure lesions were quantified by a lesion scoring system based on size and extension of lesions with larger scores assigned to the more severe lesions. Calves inoculated with live *Pasteurella* sp by aerosol or parenteral routes developed high serum antibody titers to the homologous organism, as determined by a quantitative fluorometric procedure. Mean lesion scores were approximately 2 to 20 times higher in control than those in vaccinated calves. There was a significant correlation ( $P$  less than 0.05) between high serum antibody titers at the time of challenge exposure and a low lesion score in 4 of 6 experiments.

Tags: Animal; Female; Male

Bovine pneumonic pasteurellosis: experimental induction in vaccinated  
and nonvaccinated calves.

Friend SC; Wilkie BN; Thomson RG; Barnum DA  
Canadian journal of comparative medicine (CANADA) Jan 1977, 41 (1)  
p77-83, ISSN 0008-4050 Journal Code: C10

Languages: ENGLISH

Document type: JOURNAL ARTICLE

JOURNAL ANNOUNCEMENT: 7705

Subfile: INDEX MEDICUS

A study was undertaken to investigate the effects of the immune response induced by combined aerosol and parenteral vaccination on the lung lesions induced in calves by *Pasteurella haemolytica* AI. Twenty-four calves, twelve of which had been vaccinated with killed *P. haemolytica* by aerosol and subcutaneous injection in Freund's complete and incomplete adjuvant were challenged by intratracheal inoculation of live *P. haemolytica*. Serological response to vaccination was not marked but was best measured by the whole cell agglutination test or by indirect bacterial agglutination rather than by the passive haemagglutination test. Titres of vaccinees were positively correlated with the degree of pneumonic change following challenge while in nonvaccinated controls, titres were negatively correlated with lung lesions. These findings suggest the occurrence of an immunologically mediated hypersensitivity pneumonitis in the lungs of vaccinees and point to the potential efficacy of live bacterial aerosols for stimulation of protective immunity in pneumonic pasteurellosis.

Tags: Animal

Evaluation of an oil adjuvant vaccine for control of pneumococic  
pasteurellosis in sheep

Zamri-Saad, M. Ismail, M.S.; Norizah, A.; Bahaman, A.R.; Sheikh-Omar,  
A.R.

Canberra, A.C.T. : Australian Centre for International Agricultural  
Research, 1985-

ACIAR proceedings. 1993. (43) p. 177-179.

ISSN: 1038-6920

DNAL CALL NO: S542.A8A34

Language: English

In the series analytic: Pasteurellosis in production animals / edited by  
B.E. Patten, T.L. Spencer, R.B. Johnson, D. Hoffman and L. Lehane.;  
Meeting held on August 10-13, 1993, Bali, Indonesia.

Includes references

Place of Publication: Australia

PATHOGENESIS AND IMMUNOLOGICAL ASPECTS OF BOVINE PULMONARY PASTEURELLOSIS

AUTHOR: TRIGO-T F J

AUTHOR ADDRESS: DEP. PATOLOGIA, FACULTAD MEDICINA VETERINARIA ZOOTECNIA,  
UNIVERSIDAD NACIONAL AUTONOMA MEXICO, 04510 MEXICO, D.F.

JOURNAL: VETERINARIA (MEX CITY) 22 (2). 1991. 131-134.

FULL JOURNAL NAME: VETERINARIA (Mexico City)

CODEN: VTERB

RECORD TYPE: Abstract

LANGUAGE: SPANISH

ABSTRACT: Due to the relevance of the bovine pneumonic **pasteurellosis**,  
the pathogenesis of the disease and recent advances in immunoprophylaxis  
are presented and discussed. First of all, the virus-bacteria interaction  
in the bovine lung is presented, emphasizing the relevance of the  
immunopathologic process which destroys virus-infected macrophages,  
enabling the establishment of pathogenic bacteria. Concerning the  
bacteria, the importance of the leucotoxin as a pathogenic mechanism is  
analyzed. Finally, historic and actual trends in immunogen development  
are **reviewed**, in particular the use of live **vaccines** and  
bacterial-extract biologicals.

Variation of abscess formation in cattle after vaccination with a modified-live *Pasteurella haemolytica* vaccine.

Littledike ET

USDA, Agricultural Research Service, Roman L. Hruska US Meat Animal Research Center, Clay Center, NE 68933.

American journal of veterinary research (UNITED STATES) Aug 1993, 54 (8) p1244-8, ISSN 0002-9645 Journal Code: 40C

Languages: ENGLISH

Document type: JOURNAL ARTICLE

JOURNAL ANNOUNCEMENT: 9401

Subfile: INDEX MEDICUS

During the spring of the first year of a vaccine study, 57 of 238 calves (24%), in which modified-live *Pasteurella haemolytica* vaccine (MLV) was injected twice, developed 1 or more abscesses. Abscesses were not observed after multiple visual examinations of 437 calves given killed *P. haemolytica* bacterin or placebo injections of similar adjuvants used in the vaccine and bacterin. Calves that developed abscesses after the second injection of MLV weighed significantly ( $P < 0.05$ ) less (on the basis of body weight adjusted for weaning weight) at the second injection than did those that did not develop abscesses. Compared with calves given MLV that did not develop observable abscesses, calves developing abscesses after the second injection of MLV weighed 11.0 and 14.2 kg less, respectively, at 56 days and 112 days after injection, and they had 11.0 kg less gain at 56 days after injection. Abscess prevalence tended to be highest on certain days or at certain locations used for cattle processing, and the prevalence of abscesses increased in cattle processed later on a given day. Abscesses were not observed in 2 other groups of similarly treated calves vaccinated in the autumn or in the subsequent spring.

Tags: Animal

Clinical and serological evaluation of a *Pasteurella haemolytica* A1 capsular polysaccharide vaccine.

Conlon JA; Shewen PE

Department of Veterinary Microbiology and Immunology, University of Guelph, Ontario, Canada.

Vaccine (ENGLAND) 1993, 11 (7) p767-72, ISSN 0264-410X

Journal Code: X60

Languages: ENGLISH

Document type: JOURNAL ARTICLE

JOURNAL ANNOUNCEMENT: 9311

Subfile: INDEX MEDICUS

The purified capsular polysaccharide (CPS) of *Pasteurella haemolytica* A1 was examined for its ability to protect cattle from experimental challenge with logarithmic-phase *P. haemolytica*. Several preparations of *P. haemolytica* antigens were utilized in the experiment including CPS, log-phase *P. haemolytica* culture supernatant, *P. haemolytica* recombinant leucotoxin (rLkt) and various combinations of the above. CPS alone or in combination with culture supernatant or rLkt elicited no protection; rather, administration of CPS was associated with a high incidence of anaphylaxis (36% of calves). Although a classical biphasic humoral immune response to CPS could be detected in all calves that received this compound, this T-dependent response was not correlated with resistance to experimental challenge. The complexity of protective immunity in pneumonic pasteurellosis is emphasized by this study, and clinical anaphylaxis associated with response to CPS may be implicated in the pathogenesis of disease.

**molytica A2 enhances protection against experimental pasteurellosis in lambs.**

Gilmour NJ; Donachie W; Sutherland AD; Gilmour JS; Jones GE; Quirie M  
Moredun Research Institute, Edinburgh, UK.

Vaccine (ENGLAND) Feb 1991, 9 (2) p137-40, ISSN 0264-410X

Journal Code: X60

Languages: ENGLISH

Document type: JOURNAL ARTICLE

JOURNAL ANNOUNCEMENT: 9110

Subfile: INDEX MEDICUS

A vaccine containing sodium salicylate extract (SSE) of *Pasteurella haemolytica* A2 cells grown in a medium chemically depleted of available iron by the addition of alpha alpha dipyridyl to induce iron-regulated proteins (IRPs) conferred protection to specific pathogen-free (SPF) lambs exposed to an aerosol of *P. haemolytica* A2. The disease score in these lambs was significantly lower ( $p$  less than 0.005) than those in unvaccinated lambs or in lambs immunized with SSE prepared from cells grown in iron-replete medium. Immunoblotting of sera from these SPF lambs against whole cell antigens of *P. haemolytica* A2 grown under iron-restricted conditions demonstrated that antibodies to IRPs were present only in the sera of animals immunized with SSE-IRP. The antibody profile of sera from the SSE-IRP group was similar to that obtained with serum from a lamb which had recovered from *P. haemolytica* A2 disease produced experimentally. Negligible levels of cytotoxin-neutralizing and bactericidal antibodies were detectable in the SSE-IRP group and therefore appear not to be involved in the protection observed in this experiment.

Tags: Animal; Support, Non-U.S. Gov't

**Vaccines for respiratory disease in cattle.**

Peters AR

Meat and Livestock Commission, Queensway House, Bletchley, UK.

Vaccine (ENGLAND) Sep 1987, 5 (3) p164, ISSN 0264-410X

Journal Code: X60

Languages: ENGLISH

Document type: JOURNAL ARTICLE

JOURNAL ANNOUNCEMENT: 8802

Subfile: INDEX MEDICUS

Respiratory disease is one of the most serious disease complexes affecting beef cattle production. For example, it is claimed to cost the UK industry about 70 million pounds per year. It is usually associated with young cattle and can occur in a variety of situations. It is a good example of multifactorial disease in that its aetiology involves both infection by a variety of microorganisms and a number of environmental factors. Several distinct syndromes occur and a number of microorganisms are thought to be important including the bacteria *Pasteurella haemolytica* type A1, *P. multocida*, *Haemophilus somnus*, *Corynebacterium pyogenes*, *Mycoplasma bovis* and *M. dispar*. Of the viruses, bovine herpes virus 1 (BHV1) and respiratory syncytial virus (RSV) are known to be important, the former also causing the specific syndrome, infectious bovine rhinotracheitis (IBR) in addition to its involvement in the pneumonia complex. Other viruses of possible importance include para-influenza 3 (Pi3), adenoviruses, bovine viral diarrhoea (BVD) virus, coronavirus and rhinovirus.

Preliminary studies with a live streptomycin-dependent *Pasteurella multocida* and *Pasteurella haemolytica* vaccine for the prevention of bovine pneumonic pasteurellosis.

Catt DM; Chengappa MM; Kadel WL; Herren CE

Canadian journal of comparative medicine (CANADA) Oct 1985, 49 (4)

p366-71, ISSN 0008-4050 Journal Code: CI0

Languages: ENGLISH

Document type: JOURNAL ARTICLE

JOURNAL ANNOUNCEMENT: 8604

Subfile: INDEX MEDICUS

Twelve *Pasteurella*-free Holstein-Friesian calves were used in a study to test the efficacy of a live streptomycin-dependent *Pasteurella multocida* A:3 and streptomycin-dependent *Pasteurella haemolytica* A1 vaccine. The calves were inoculated intramuscularly twice at 14-day intervals with either the streptomycin-dependent vaccine, containing  $1 \times 10^{(6)}$  colony forming units/mL *P. multocida* and  $4 \times 10^{(8)}$  colony forming units/mL *P. haemolytica*, commercial bacterin, or phosphate buffered saline. Two weeks following the second vaccination, all calves were challenged by intranasal inoculation of  $10^{(8)}$  TCID<sub>50</sub>/4.0 mL infectious bovine rhinotracheitis virus followed three days later by intratracheal injection with  $2.3 \times 10^{(7)}$  colony forming units/mL of a 16 hour culture of *P. multocida* A:3 and  $2.6 \times 10^{(8)}$  colony forming units/mL of an 8 hour culture of *P. haemolytica* A1. Seven days after challenge with

*Pasteurella*, calves were killed for collection of tissues at necropsy. Each calf was given a score based on macroscopic and microscopic lesions. The scores for the calves receiving live vaccines were significantly lower (*p* less than 0.025) than those for the controls. Also, the calves receiving live vaccines had a significant (*p* less than 0.05) increase in the level of serum antibody to *P. haemolytica*. The results of this preliminary study showed that the streptomycin-dependent vaccine offered better protection than the commercial bacterin against a virulent homologous challenge.

Tags: Animal; Male

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<u>DB Name</u>	<u>Query</u>	<u>Hit Count</u>	<u>Set Name</u>
USPT	attenuat\$ same pasteur\$	64	<u>L18</u>
USPT	l16 not l11	11	<u>L17</u>
USPT	l15 and (leukotox\$ or leuko-toxi\$)	22	<u>L16</u>
USPT	(attenuat\$ or inactivat\$ or mutagen\$ or mutan\$ or mutation\$) same pasteurell\$	105	<u>L15</u>
USPT	l13 not l11	0	<u>L14</u>
USPT	l12 same (l11 or l10)	25	<u>L13</u>
USPT	(dna or mutation or mutant or mutagen\$ or cdna or rna or clone or cloned or cloning or transformed or attenuate)	137321	<u>L12</u>
USPT	lkta or lkbt or lktc or lktd	29	<u>L11</u>
USPT	lktcabd	1	<u>L10</u>
USPT	ltkcabd	0	<u>L9</u>
USPT	l7 and (dna or mutation or mutant or mutagen\$ or cdna or rna or clone or cloned or cloning or transformed or attenuate)	37	<u>L8</u>
USPT	l6 and hemoly\$	40	<u>L7</u>
USPT	leukotoxin	64	<u>L6</u>
USPT	ctxcabd	0	<u>L5</u>
USPT	ctxcabd	0	<u>L4</u>
USPT	ctcabd	0	<u>L3</u>
USPT	cabd	27	<u>L2</u>
USPT	cktcabd	0	<u>L1</u>

*file*

Deletion analysis resolves cell-binding and lytic domains of the *Pasteurella* leukotoxin.

Cruz WT; Young R; Chang YF; Struck DK

Department of Medical Biochemistry and Genetics, College of Medicine,  
Texas A & M University, College Station 77843.

Mol Microbiol (ENGLAND) Nov 1990, 4 (11) p1933-9, ISSN 0950-382X

Journal Code: MOM

Languages: ENGLISH

Document type: JOURNAL ARTICLE

JOURNAL ANNOUNCEMENT: 9107

Subfile: INDEX MEDICUS

A series of internal deletions in the lktA gene of *Pasteurella* haemolytica has been constructed. All of the deletions eliminated the lytic activity of the leukotoxin towards the bovine lymphoma cell line, BL-3. Deletions removing segments of the amino-proximal hydrophobic region, which is thought to constitute an essential membrane-spanning domain, were found to agglutinate BL-3 cells. Agglutination was similar to lysis by the wild-type toxin in that it was dependent upon the presence of calcium and required expression of the lktC gene. The agglutinating deletion proteins protected BL-3 cells from lysis by the wild-type toxin in a competitive fashion. This suggests that these mutants bind to a surface feature of the leukocyte which interacts with the native leukotoxin. These findings demonstrate that the cell-binding and lytic domains of the leukotoxin are separable.

Tags: Animal; Support, Non-U.S. Gov't; Support, U.S. Gov't, Non-P.H.S.

Descriptors: Bacterial Toxins--Genetics--GE; \*Chromosome Deletion;

\*Exotoxins--Genetics--GE; \*Genes, Bacterial; \**Pasteurella* --Genetics--GE;  
Cattle; Cell Line; Cell Survival--Drug Effects--DE; Codon--Genetics--GE;  
Exotoxins--Metabolism--ME; Exotoxins--Pharmacology--PD; Lymphoma;  
Mutagenesis, Site-Directed; *Pasteurella*--Metabolism--ME; Plasmids

CAS Registry No.: 0 (leukotoxin); 0 (Bacterial Toxins); 0 (Codon);  
0 (Exotoxins); 0 (Plasmids)

product of *Azotobacter vinelandii*, and that of ORF4 was similar to the algP product of *P. aeruginosa* and to eukaryotic histone H1 proteins. The proteins of ORF2 and ORF3 appear to be previously unidentified.

Tags: Support, Non-U.S. Gov't

Descriptors: \*Cloning, Molecular; \*Genes, Bacterial; \*Pseudomonas aeruginosa--Genetics--GE; Acyltransferases--Chemistry--CH; Acyltransferase s--Genetics--GE; Alcaligenes--Genetics--GE; Amino Acid Sequence; Base Sequence; Blotting, Northern; Carboxylic Ester Hydrolases--Chemistry--CH; Carboxylic Ester Hydrolases--Genetics--GE; Chromosome Mapping; Gene Expression; Molecular Sequence Data; Operon; Polyesters--Metabolism--ME; Pseudomonas--Genetics--GE; Restriction Mapping; Transcription, Genetic Molecular Sequence Databank No.: GENBANK/X66592; GENBANK/Z11975; GENBANK/S42403; GENBANK/S42404; GENBANK/M85175; GENBANK/M85176; GENBANK/M85177; GENBANK/X65179; GENBANK/X65180; GENBANK/X65181

CAS Registry No.: 0 (Polyesters)

Enzyme No.: EC 2.3. (Acyltransferases); EC 2.3.1.- (poly(3-hydroxyalkanoic acid) synthase); EC 3.1.1 (Carboxylic Ester Hydrolases); EC 3.1.1.- (poly(3-hydroxyalkanoic acid) depolymerase)

Gene Symbol: phaC1Pa; phaC2Pa; phaDPa

4/9/63 (Item 5 from file: 155)

DIALOG(R) File 155: MEDLINE(R)

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06804347 92040099

**Identification of RTX toxin target cell specificity domains by use of hybrid genes.**

Forestier C; Welch RA

Department of Medical Microbiology and Immunology, University of Wisconsin-Madison 53706.

Infection and immunity (UNITED STATES) Nov 1991, 59 (11) p4212-20,  
ISSN 0019-9567 Journal Code: GO7

Contract/Grant No.: AI20323, AI, NIAID

Languages: ENGLISH

Document type: JOURNAL ARTICLE

JOURNAL ANNOUNCEMENT: 9202

Subfile: INDEX MEDICUS

The *Escherichia coli* hemolysin (HlyA) and *Pasteurella haemolytica* leukotoxin (LktA) are cytoytic toxins encoded by genes belonging to the recently described RTX gene family. These cytotoxins are, respectively, 1,023 and 953 amino acids in length and are encoded by genes within identically organized operons. They share 45% amino acid sequence identities but differ in their target cell specificities. In vitro-derived recombinant hybrid genes between hlyA and lktA were constructed by using restriction endonuclease sites created by oligonucleotide site-directed mutagenesis. The cytoytic activity of hybrid proteins was investigated using as targets sheep erythrocytes and two cultured cell lines from different species (BL3, bovine leukemia-derived B lymphocytes; and Raji, human B-cell lymphoma cells). HlyA is cytoytic to all three cell types. LktA lyses only BL3 cells. Among the hybrid proteins displaying cytoytic activity, the striking finding is that the hemolytic activity of several LktA-HlyA hybrids was independent of any cytoytic activity against either cultured cell species. The hemolytic activity was associated with the HlyA region between amino acids 564 and 739. Structures that are critical for HlyA cytoytic activity against BL3 or Raji cells were destroyed when LktA-HlyA and HlyA-LktA hybrids were made, respectively, at amino acid positions 564 and 739 of HlyA. In contrast to HlyA, which lysed the two different cultured cell lines with equal efficiency, Lkt-HlyA hybrids possessing the amino-terminal 169 residues of LktA lysed BL3 cells more efficiently than Raji cells. This suggests that a significant but not exclusive element of the LktA ruminant cell specificity resides in the amino-terminal one-fifth of the protein. A molecular model of the functional domains of HlyA and LktA is presented.

Tags: Comparative Study; Human; In Vitro; Support, Non-U.S. Gov't; Support, U.S. Gov't, Non-P.H.S.; Support, U.S. Gov't, P.H.S.

Descriptors: Cytotoxins--Toxicity--TO; \*Escherichia coli--Pathogenicity

--PY; \*Exotoxins--Toxicity--TO; \*Hemolysins--Toxicity--TO; \***Pasteurella haemolytica**--Pathogenicity--PY; Amino Acid Sequence; Bacterial Toxins--Chemistry--CH; Bacterial Toxins--Genetics--GE; Bacterial Toxins--Toxicity--TO; Base Sequence; Cell Death; Cells, Cultured; Cytotoxins--Chemistry--CH; Cytotoxins--Genetics--GE; DNA Mutational Analysis; Exotoxins--Chemistry--CH; Exotoxins--Genetics--GE; Hemolysins--Chemistry--CH; Hemolysins--Genetics--GE; Hemolysis; Molecular Sequence Data; Oligonucleotides--Chemistry--CH; Recombinant Fusion Proteins; Structure-Activity Relationship  
CAS Registry No.: 0 (leukotoxin); 0 (Bacterial Toxins); 0 (Cytotoxins); 0 (Exotoxins); 0 (Hemolysins); 0 (Oligonucleotides); 0 (Recombinant Fusion Proteins)  
Gene Symbol: hlyA; lktA

4/9/64 (Item 6 from file: 155)

DIALOG(R)File 155: MEDLINE(R)

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06560999 91186829

Deletion analysis resolves cell-binding and lytic domains of the **Pasteurella** leukotoxin.

Cruz WT; Young R; Chang YF; Struck DK  
Department of Medical Biochemistry and Genetics, College of Medicine, Texas A & M University, College Station 77843.

Molecular microbiology (ENGLAND) Nov 1990, 4 (11) p1933-9, ISSN 0950-382X Journal Code: MOM

Languages: ENGLISH

Document type: JOURNAL ARTICLE

JOURNAL ANNOUNCEMENT: 9107

Subfile: INDEX MEDICUS

A series of internal deletions in the lktA gene of **Pasteurella haemolytica** has been constructed. All of the deletions eliminated the lytic activity of the **leukotoxin** towards the bovine lymphoma cell line, BL-3. Deletions removing segments of the amino-proximal hydrophobic region, which is thought to constitute an essential membrane-spanning domain, were found to agglutinate BL-3 cells. Agglutination was similar to lysis by the wild-type toxin in that it was dependent upon the presence of calcium and required expression of the lktC gene. The agglutinating deletion proteins protected BL-3 cells from lysis by the wild-type toxin in a competitive fashion. This suggests that these mutants bind to a surface feature of the leukocyte which interacts with the native **leukotoxin**. These findings demonstrate that the cell-binding and lytic domains of the **leukotoxin** are separable.

Tags: Animal; Support, Non-U.S. Gov't; Support, U.S. Gov't, Non-P.H.S.

Descriptors: Bacterial Toxins--Genetics--GE; \*Chromosome Deletion; \*Exotoxins--Genetics--GE; \*Genes, Bacterial; \***Pasteurella** --Genetics--GE; Cattle; Cell Line; Cell Survival--Drug Effects--DE; Codon--Genetics--GE; Exotoxins--Metabolism--ME; Exotoxins--Pharmacology--PD; Lymphoma; Mutagenesis, Site-Directed; **Pasteurella** --Metabolism--ME; Plasmids

CAS Registry No.: 0 (leukotoxin); 0 (Bacterial Toxins); 0 (Codon); 0 (Exotoxins); 0 (Plasmids)

Gene Symbol: lktA

4/9/65 (Item 7 from file: 155)

DIALOG(R)File 155: MEDLINE(R)

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06558817 91178497

Time and temperature dependence of granulocyte damage by leucotoxic supernatants from **Pasteurella haemolytica** A1.

Styrt B; Walker RD; Dahl LD; Potter A  
Department of Medicine, Michigan State University, East Lansing 48824.  
Journal of general microbiology (ENGLAND) Nov 1990, 136 ( Pt 11) p2173-8, ISSN 0022-1287 Journal Code: I87

Languages: ENGLISH

Document type: JOURNAL ARTICLE

JOURNAL ANNOUNCEMENT: 9107

Subfile: INDEX MEDICUS

Bacterial exotoxins may contribute to the pathogenic potential of micro-organisms through interactions with cells of the host defence system as well as by directly damaging host tissue. The present studies were designed to explore mechanisms of interaction between bovine granulocytes and the leucotoxin produced by *Pasteurella haemolytica*, a major cause of bovine respiratory disease. Leucotoxin-containing supernatant from *P. haemolytica* A1 caused rapid cell death in isolated bovine granulocytes that was close to half-maximal by 5 min and nearly 90% complete after 30 min at 37 degrees C. Maintaining granulocytes at ice-water temperature markedly attenuated or prevented the toxic effect; furthermore, if exposed to supernatants at ice-water temperature and then washed, most cells remained viable even after rewarming to room temperature. However, even a very brief exposure (about 5 s) at 37 degrees C led to extensive cell death even after immediate cold dilution and washing. Granule enzymes such as arylsulphatase were released far more slowly than cytosol contents. Leucotoxin purified by column chromatography showed temperature dependence and divergence between cytosol and granule marker release similar to those observed with the crude supernatant preparation. These findings indicate that irreversible interaction between *P. haemolytica* leucotoxin and bovine granulocytes is initiated very rapidly at 37 degrees C but markedly impeded at low temperature, while granule enzyme release follows cytosol marker release over a much longer period. The results suggest either a requirement for target cell metabolic activity to initiate toxin effects or a temperature-dependent receptor conformation, with granule enzyme release following as a secondary consequence of granulocyte death.

Tags: Animal; Support, Non-U.S. Gov't; Support, U.S. Gov't, Non-P.H.S.

Descriptors: Exotoxins--Pharmacology--PD; \*Granulocytes--Drug Effects--DE ; \**Pasteurella*--Analysis--AN; Arylsulfatases--Metabolism--ME; Biological Markers; Cattle; Cell Survival; Cytosol--Enzymology--EN; Exotoxins --Isolation and Purification--IP; Granulocytes--Enzymology--EN; Lactate Dehydrogenase--Metabolism--ME; Temperature

CAS Registry No.: 0 (leukotoxin); 0 (Biological Markers); 0 (Exotoxins)

Enzyme No.: EC 1.1.1.27 (Lactate Dehydrogenase); EC 3.1.6.1 (Arylsulfatases)

4/9/66 (Item 8 from file: 155)

DIALOG(R) File 155: MEDLINE(R)

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05854975 90036680

Regulation of expression of the *Pasteurella haemolytica* leukotoxin determinant.

Strathdee CA; Lo RY

Department of Microbiology, University of Guelph, Ontario, Canada.

Journal of bacteriology (UNITED STATES) Nov 1989, 171 (11) p5955-62,

ISSN 0021-9193 Journal Code: HH3

Languages: ENGLISH

Document type: JOURNAL ARTICLE

JOURNAL ANNOUNCEMENT: 9002

Subfile: INDEX MEDICUS

The *Pasteurella haemolytica* leukotoxin determinant is composed of four contiguous genes encoded on the same DNA strand and denoted lktCABD, in the order of their genetic organization. To gain a better understanding of the expression and regulation of the leukotoxin, the transcripts and promoters of the lkt determinant were mapped. Northern (RNA) blot analysis revealed two sets of transcripts. One set was 3.7 and 3.4 kilobases long, encoded lktCA, and comprised approximately 90% of the transcripts, whereas the other set was 7.4 and 7.1 kilobases long and encoded lktCABD. Two promoters were present, and each had features similar to the *Escherichia coli* consensus promoter sequences. Both promoters were located upstream from lktC; they were separated by 258 base pairs, as mapped by primer extension analysis. These results suggest a mechanism of expression similar

to that of the related *E. coli* hemolysin. Transcription initiated upstream from lktC at either promoter and continued through lktC and lktA to a rho-independent transcriptional termination signal in the lktA-lktB intercistronic region. This signal attenuated expression by terminating 90% of transcription to generate the 3.7- and 3.4-kilobase lktCA transcripts. The remaining readthrough transcription generated full-length 7.4- and 7.1-kilobase lktCABD transcripts. Expression of the leukotoxin was greatly reduced by growth at 30 degrees C, pH 6.5, and Fe<sup>2+</sup> limitation. These conditions also modulated the expression of a number of other secreted proteins, which suggests that all of these secreted proteins are controlled by the same regulatory mechanism.

Tags: Support, Non-U.S. Gov't

Descriptors: Bacterial Toxins--Genetics--GE; \*Exotoxins--Genetics--GE; \*Gene Expression; \*Gene Expression Regulation, Bacterial; \*Genes, Structural, Bacterial; \* *Pasteurella* --Genetics--GE; \*Transcription, Genetic; Base Sequence; Blotting, Northern; Molecular Sequence Data; Plasmids; Restriction Mapping; RNA, Bacterial--Genetics--GE; RNA, Bacterial--Isolation and Purification--IP

Molecular Sequence Databank No.: GENBANK/M30793

CAS Registry No.: 0 (leukotoxin); 0 (Bacterial Toxins); 0 (Exotoxins); 0 (Plasmids); 0 (RNA, Bacteria)